Tocopherol Oxidation in Natural Fats

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Destruction of tocopherols during autoxidation of fats was studied to gain more information about the mechanism of their antioxidant action. Tocopherol loss during autoxidation was much smaller in the highly unsaturated vegetable oils than in cottonseed oil and lard. Metal contaminants increase appreciably the extent of tocopherol oxidation. Their effect is eliminated by the addition of 0.01% citric acid. The work suggests that the hydroperoxides formed in the highly unsaturated vegetable oils decompose rapidly before they react with tocopherol. Antioxidants that react more rapidly than tocopherol with polyunsaturated fat hydroperoxides are needed to stabilize highly unsaturated vegetable oils.

THE MARKED DIFFERENCE in autoxidative behavior between vegetable and animal fats has generally been attributed to the relatively high concentration of tocopherols in vegetable fats. Relatively little work has been published on the fate of these natural antioxidants during the autoxidation of vegetable oils. Early studies of Golumbic (*10, 11*) with ethyl esters of lard and hydrogenated cottonseed oil, as well as those of Swift, Rose, and Jamieson (*23*) with methyl esters of cottonseed oil, have shown that tocopherol is completely destroyed by the end of the induction period. However, in these studies and in a more recent one by Luckmann and Melnick (*15*), the interference of peroxides with the determination of tocopherol in the oxidized fats was not considered. Oliver, Singleton, and Bailey (*17*) used the sulfuric acid treatment of Parker and McFarlane (*18*) to remove interfering peroxides. They found that in lard containing 0.5% of α-tocopherol or peanut oil antioxidants, an appreciable concentration of the initial tocopherol (10 to 20%) remained in the rancid fats at the end of the induction period. The concentration of tocopherol added by these authors is abnormally high and may constitute a pro-oxidant level of antioxidant.

The interference of peroxides and other substances in oxidized fats and methyl esters of fatty acids with tocopherol determination has received more careful attention recently (*7, 8, 13*). With the development of adequate procedures for the removal of interfering substances the problem of determining the rate of tocopherol loss during autoxidation of fats was opened for serious study. Lips (*14*) found that the rate of destruction of tocopherol, expressed as tocopherol half life, increased with the degree of unsaturation of the autoxidizing methyl esters of fatty acids. At the end of the induction period less than 15% of the tocopherol remained in the oleate and stearate, whereas up to 50% of the tocopherol remained in the linoleate and linolenate. The rates of tocopherol destruction in this study were considered too complicated to be treated kinetically.

In a previous paper (*8*) it was reported that the loss of tocopherol observed in different oxidized soybean oils at the end of the induction period was less than 10%. The present work was undertaken to examine primarily the loss of natural tocopherols in different vegetable oils as compared to the loss of α-tocopherol added to lard. Particular attention was given to the relative initial rates of tocopherol loss which are important in the ranges of oxidation pertinent to the edible fat field. The strik-

Table I. Rate of Tocopherol Loss in Autoxidizing Fats

Fat Samples[a]	Peroxide Induction Period, Hr.	Peroxide Value at End of Induction Period, Meq./Kg.	Initial Rates[b]	
			Peroxide development, meq./kg./hr.	Tocopherol oxidation, γ/g./hr.
Part A				
Oxidation at 60° C., Warburg apparatus				
Soybean oil A				
1530 γ/gram tocopherol	16	1.7	0.10	3.5
+0.01% citric acid	105	2.4	0.02	1.4
+0.3 p.p.m. Fe^{+++}	4	1.7	0.41	43
+0.3 p.p.m. Fe^{+++} + 0.01% citric acid	100	2.0	0.02	1.5
Soybean oil B, 1440 γ/gram tocopherol	20	3.0	0.15	3.3
Stripped soybean oil A[c]				
810 γ/gram tocopherol	50	2.7	0.05	2.2
324 γ/gram tocopherol	40	3.4	0.06	1.3
Oxidation at 100° C., A.O.M. conditions				
Soybean oil A	5	3.0	0.60	4.9
+0.01% citric acid	6	1.9	0.31	4.6
+0.3 p.p.m. Fe^{+++}	0	...	8.2	32
Part B				
Oxidation at 60° C., Warburg apparatus				
Safflower oil A, 556 γ/gram tocopherol	6	3.5	1.0	3.9
B, 590 γ/gram tocopherol	2	0.7	0.35	3.1
Linseed oil 1060 γ/gram tocopherol	10	9.9	1.1	1.1
Lard A + 1500 γ/gram α-*d*-tocopherol	264	110	0.79	12[d]
+1465 γ/gram soybean tocopherol[e]	600	130	0.21	18[d]
Oxidation at 100° C., A.O.M. conditions				
Cottonseed oil A, 910 γ/gram tocopherol	6	28	4.7	67
+0.01% citric acid	5	2.2	0.44	11
Corn oil A, 1320 γ/gram tocopherol	2	1.4	0.72	21
+0.01% citric acid	10	5.1	0.51	5.1
Lard A + 500 γ/gram α-*d*-tocopherol	12	45	3.8	45[d]
+1500 γ/gram	8	56	7.0	180[d]
+1500 γ/gram + 0.01% citric acid	27	140	4.1	23
Lard B + 1460 γ/gram α-*d*-tocopherol	17	85	5.7	84

[a] Iodine values (Wijs) were: soybean oils A and B: 127, 129; safflower oils A and B: 149, 145; linseed oil: 192; lards A and B: 68.6, 66.5; cottonseed oil: 102.4; corn oil: 126.
[b] Initial rates refer to changes occurring during induction.
[c] Oils treated with carbon black to remove part of natural tocopherols (*8*).
[d] Rates during initial period only because tocopherol oxidation follows first-order kinetics.
[e] Soybean tocopherols added in form of concentrate containing 60% tocopherols.

ing effect of metal contaminants was also investigated.

Experimental

Extracted and refined vegetable oils and lard were obtained from various commercial sources. They were deodorized for 3 hours in vacuum, in a laboratory all-glass apparatus at 210° C. In accelerated oxidation tests, reliable and reproducible results could be obtained only when the initial peroxide value of the samples was 0. The oil samples were therefore redeodorized on a small scale for 15 minutes immediately before starting each oxidation test.

Oxidations were carried out at 60° and 100° C. Oxidation at 60° C. was done in a Warburg apparatus with 2.0-gram samples in 25-ml. Erlenmeyer flasks which fitted directly on the Warburg manometers. Oxidation at 100° C. was under the conditions of the active oxygen method (A.O.M.). Periodically during oxidation, 2-gram samples were removed, frozen over solid carbon dioxide, and stored in a −18° C. room until analyzed. Additives were incorporated into the oils from solutions in absolute alcohol or purified pentane, and the solvent was removed on a rotating evaporator, followed by deodorization for 15 minutes prior to oxidation.

Oxidation was followed by determination of peroxides by the ferric-thiocyanate method of Hills and Thiel (*12*) with 20-mg. samples of oils. This method was chosen in preference to the iodometric method, because of its greater sensitivity at the micro range. The values obtained by the ferric-thiocyanate method were approximately twice as high as those with the iodometric method. Tocopherol was determined in the oxidized samples by the Emmerie-Engel method after preliminary removal of interfering peroxides by heating (*8*).

Results

The kinetics of oxidation of tocopherol were studied in different autoxidizing fats. Preliminary results indicated that the kinetics of the initial rates of tocopherol loss were zero-order in soybean oil and first-order in lard. Tocopherol loss at the end of the induction period was considerably greater in lard (75%) than in soybean oil (2.7%) under A.O.M. conditions of oxidation. This striking difference in tocopherol loss during autoxidation was subsequently found to be partly attributable to the relative metal contamination of the fats. It became, therefore, necessary to study the relationship of trace metals and chelating agent to the tocopherol disappearance in oils.

The effect of added ferric chloride and citric acid was investigated in soybean oil. The results (Table I, Part A, and Figure 1*A*) show that the addition of

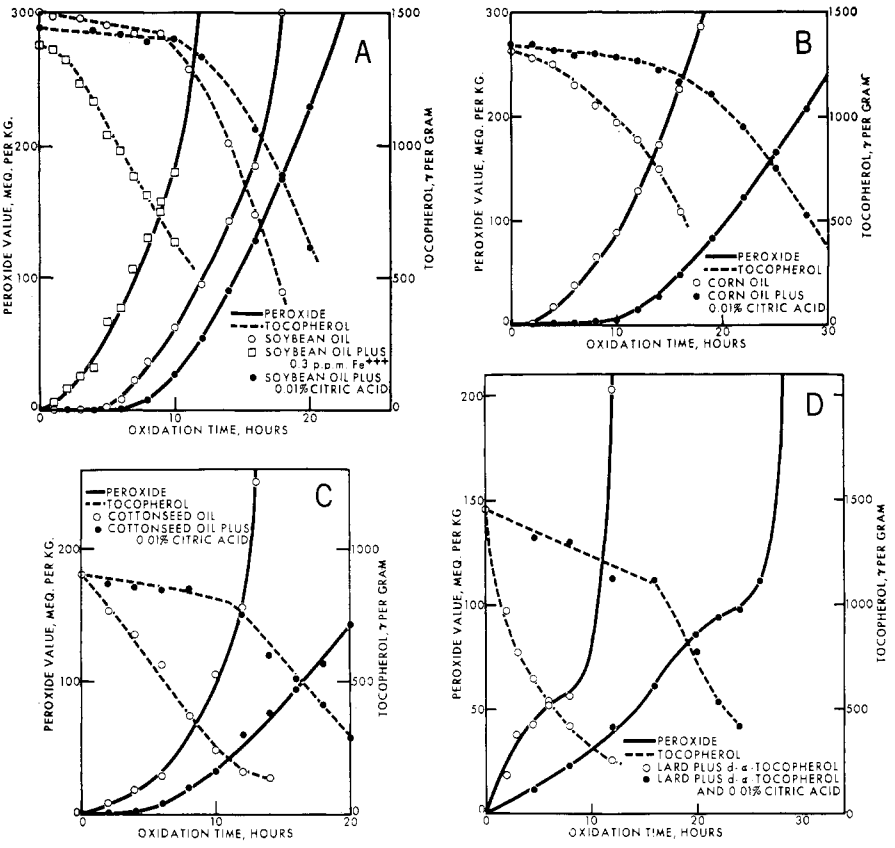

Figure 1. Effect of added iron and citric acid on tocopherol oxidation in natural fats autoxidized at 100° C. under A.O.M. conditions

A. Soybean oil
B. Corn oil
C. Cottonseed oil
D. Lard

0.3 p.p.m. of iron(III) increased appreciably the initial rate of tocopherol loss incurred during autoxidation of soybean oil with a corresponding decrease in the oxidative stability of the oil. At 60° C. the addition of 0.01% citric acid to the oil increased its oxidative stability markedly, and reduced the initial rate of tocopherol loss. The addition of both iron and citric acid gave the same results as those obtained with citric acid alone, indicating that 0.01% citric acid was sufficient to inactivate any metal contaminants. At 100° C. the addition of 0.01% citric acid increased the stability only slightly (Figure 1, *A*). In the presence of added iron and citric acid, the oil showed little change in stability (induction period, 7 hours, curve not shown).

These results were taken as a basis for the use of citric acid addition in order to compare the fate of tocopherol in different fats without the complicating effect of metal contaminant.

The tocopherol oxidation in the presence and absence of citric acid was studied during the autoxidation of soybean oil, corn oil, cottonseed oil, and lard. The results obtained are represented in Figure 1.

In the presence of citric acid, tocopherol oxidation follows the kinetics of a chain reaction (*3*) with a distinct induction period during which the rate follows zero-order kinetics. In this series, the initial rate of tocopherol loss during the induction period decreases with the degree of unsaturation of the fats (Table I). The end of the peroxide induction period, expressed as the time interval before oxidation proceeds rapidly, occurs at lower peroxide values in soybean and corn oils than in cottonseed oil or lard. It is difficult to relate the length of the induction period of tocopherol oxidation to that of the peroxide development, because the shape of the peroxide curve varies with the type of fat. The length of the tocopherol induction period increases with saturation of the fats. The effect of metal contaminants becomes apparent in fats in the absence of citric acid: The tocopherol oxidation proceeds rapidly without an induction period and the peroxide development is correspondingly faster. Without citric acid, tocopherol oxidation in soybean and corn oils proceeds autocatalytically, whereas in lard the tocopherol oxidation follows first-order kinetics (Figure 2). In cottonseed oil tocopherol oxidation approaches first-order kinetics.

The relative rates of tocopherol oxidation in different vegetable oils and lard are summarized in Table I. Removal of part of the natural tocopherols from

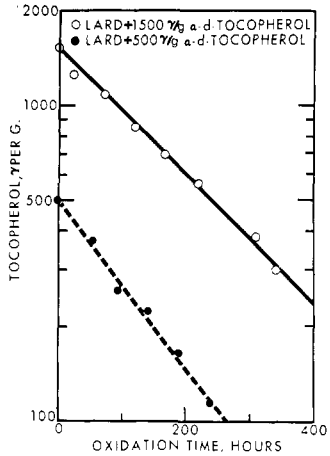

Figure 2. Kinetics of tocopherol oxidation in lard autoxidized at 60° C. in a Warburg apparatus

soybean oil by a carbon black treatment (*8*) did not affect appreciably the initial rate of loss of tocopherol during autoxidation. Therefore the initial concentration of tocopherol does not appear to affect the rate of tocopherol oxidation. This effect is to be expected in a zero-order reaction. Stripped soybean oils show greater oxidative stability when compared to the control oil. The tocopherol level of the stripped oils appears to be near to the optimum for oxidative stability. The natural level of tocopherol in soybean oil may therefore be excessive for optimum stability.

The rate of tocopherol oxidation in safflower oil and linseed oil is low and corresponds to that in soybean oil. With lard the initial tocopherol concentration affected the rates of tocopherol oxidation because it followed first-order kinetics in the absence of citric acid. The rate of oxidation of mixed soybean tocopherols in lard was of the same order of magnitude as that of α-*d*-tocopherol.

Discussion

Two principal reactions may be considered to control the fate of tocopherol in autoxidizing fats: the reaction between tocopherol and reactive fat hydroperoxides, and the spontaneous oxidation of tocopherol by atmospheric oxygen. The first reaction has been generally accepted to occur in fats, although evidence is meager. The kinetics of a chain reaction, obtained in this study, support a free radical mechanism for this first reaction. The second reaction may be unimportant, because of the relatively high stability of tocopherol as observed in inert solvents (*14*).

Swift (*22*) was among the first to show that purified hydroperoxides are effective in appreciably decreasing the oxidative stability of methyl oleate in the presence of α-tocopherol. He found that the concentration of tocopherol at the end of the induction period was zero. On the other hand, the addition of decomposed hydroperoxides had little or no effect on the stability of the ester and, presumably, on the tocopherol oxidation. The results of Dubouloz, Laurent, and Jouve (*4*) are in harmony with those of Swift. They found that tocopherol, when added to ethyl oleate during oxidation, caused a much greater peroxide destruction in a freshly oxidized ester than when the same oxidized ester was allowed to stand at room temperature for varying times. Decomposition of peroxides on standing would thus reduce their reactivity with tocopherol. Those studies are further supported by the work of Privett and Quackenbush (*19*) who showed that lard peroxides decomposed in the presence of tocopherol, in the absence of air, without affecting the tocopherol concentration. Therefore, the decomposition products of peroxides do not appear to react with tocopherol.

The results of the present study suggest that tocopherol oxidation may be related to the stability of fat hydroperoxides. The initial peroxide accumulation in highly unsaturated vegetable oils is low with correspondingly little tocopherol disappearance during the initial autoxidation. This fact could be attributed to the relative ease by which polyunsaturated fat hydroperoxides decompose (*9*). These decomposition products do not appear reactive with tocopherol. This small loss of tocopherol is particularly notable in linseed oil and soybean oil, which contain linolenic acid.

The effect of metals has been generally ignored in studies of tocopherol oxidation. The catalytic activity of a hydroperoxide-metal complex has received experimental support recently (*1, 2, 24*) to explain the pro-oxidant effect of trace metals on fat autoxidation. The profound effect of iron observed in this study may be related to the reactivity of the hydroperoxide-metal complex with tocopherol during autoxidation. The effect of organic acids like citric and ascorbic in sparing tocopherol oxidation has been demonstrated by the early work of Golumbic and Mattill (*10, 11*). In this work the antioxidant synergism of organic acids is attributed to a regeneration of the tocopherol after its oxidation by fat peroxides. Privett and Quackenbush (*20*) recently advanced a different mechanism based on the inhibition of the accelerating effect of tocopherol on the decomposition of fat peroxides. None of these workers, however, took into consideration the pronounced metal-activating effect of these organic acids in autoxidizing fats. That the antioxidant synergism of organic acids can be adequately explained by their metal-chelating effect has been shown by the previous studies of Dutton, Schwab, Moser, and Cowan (*5*), Morris, Myers, Kip, and Riemenschneider (*16*), Evans, Cooney, Moser, and Schwab (*6*).

The results in the present study show that tocopherol oxidation proceeds in the presence of citric acid and added metal at the same rate as in the absence of added metal. These results further demonstrate that the sparing action of citric acid for tocopherol oxidation can be adequately explained by its metal-inactivating effect. Because metals are known to accelerate peroxide decomposition (*1, 21*), inhibition by citric acid of the catalysis of peroxide decomposition by high concentration of tocopherol observed by Privett and Quackenbush (*19, 20*) can also be explained by the metal-inactivating effect of citric acid.

The stabilization afforded by added antioxidants to edible fats has been generally followed by peroxide and oxygen absorption measurements. A more useful index of the effectiveness of an antioxidant may be to measure its concentration during autoxidation. The relation between antioxidant oxidation and stabilization of fats needs to be established.

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CORROSION IN FOOD CONTAINERS

The Mechanism of Corrosion of Tin Plate by Various Food Products

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The corrosion mechanism of tin plate under conditions duplicating normal canned-food packs was studied. In grapefruit and tomato juice, the dissolution of the tin coating is accompanied by the evolution of hydrogen; in prunes, hydrogen does not appear to be evolved when the tin dissolves, but only when the steel base is attacked. In prunes, all tin plates detin at the same rate, but in grapefruit and tomato juice the rate of detinning is not the same for different types of tin plate. Electrochemical data indicate the presence of depolarizers in prunes, but not in grapefruit juice, a fact that explains the different corrosion mechanisms in the various food products.

THE CORROSION MECHANISM of tin plate by various food products has been the subject of extensive investigations by the steel industry and the can-manufacturing industry. In the late 1920's, various groups showed that in deoxygenated acid-food products, tin is anodic to the steel base and protects it at its own expense (*4–6*). The next 15 years were spent primarily in investigating the effect of different manufacturing variables on the corrosion performance of tin plate (*2*). In recent years, work has been directed toward a study of the corrosion mechanism and the interrelation of the three reactions that occur in the corrosion process: dissolution of the tin, dissolution of the steel base, and evolution of hydrogen (*1, 3, 7*). Most of this work has been performed with prunes in water as an accelerated corrosion medium.

The purpose of the research reported was to determine whether the corrosion mechanism of tin plate is the same in all food products as in prunes.

Experimental

The three food products used as corrosive media were prunes in water, grapefruit juice, and tomato juice. They were packed in No. 2 (307 × 409) cans, made from 0.50 pound per base box (a trade unit; 1 pound per base box equals 454 grams of tin per 201,272 sq. cm. of steel surface) tin plate according to normal canning procedures, and then stored at 37.8° C. Therefore, except for the high temperature storage, this pack simulated a normal commercial pack. The volume of the corrodant was approximately 570 ml., and the surface area exposed to it was approximately 310 sq. cm. This area was covered with a layer of tin 0.65 micron thick. This quantity of tin is equal to 2.5 meq. of stannous tin per can. Between the tin and the steel base was an iron-tin compound, $FeSn_2$, that was approximately 0.15 micron thick.

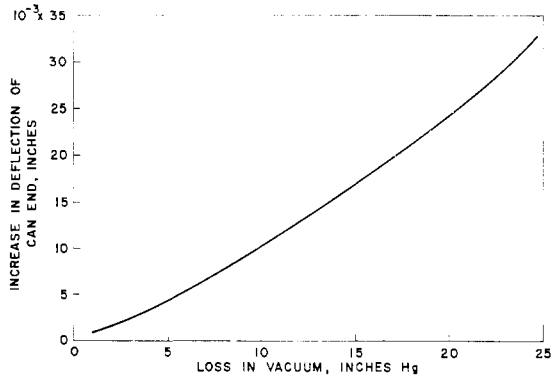

Figure 1. Change in dial reading vs. change in internal pressure

To follow the corrosion kinetics, data were obtained for tin and iron dissolution and hydrogen evolution as a function of time. Because data on the tin and iron contents of the food products could be obtained only by destructive methods, a group of cans was set aside specifically for that purpose. Three to five cans were opened at predetermined intervals and the contents were analyzed for tin and iron. The results were expressed in terms of milliequivalents of stannous tin and ferrous iron. The rate of change of hydrogen content was determined by observing the change in the contour of the can end. The ends of a can behave like a diaphragm, in that they deflect with change in internal pressure. A micrometer dial-depth gage permits measurement of this deflection, the double seam being used as a reference. The relationship between the change in the deflection and the change in the pressure in the can is shown in Figure 1.

The data obtained for the hydrogen pressure in the above manner were converted to milliequivalents of hydrogen by using the ideal gas law and assuming that the head space of the cans averaged 40 ml. This is an average value for commercially packed cans and can vary over wide limits, inasmuch as 1 mm. of head space is equivalent to 6.3 ml. of volume.

The calculation of the evolved hydrogen contained numerous assumptions, —e.g., all the evolved gas was hydrogen; none of the hydrogen dissolved in the corroding solution, food product, or steel base or diffused through the can.

With respect to the first assumption, analyses of the head space gas indicate that some carbon dioxide is also given off, but the quantity is small compared to the quantity of evolved hydrogen. The last two assumptions are also not completely valid. The resultant error, however, is in the direction opposite to that of the first assumption, so that the errors tend to cancel. Furthermore, the results, if not quantitative, show very definite qualitative trends.

Results and Discussion

The milliequivalents of the three corrosion products are plotted as a function of time for the corrosion of one